PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of)
Jevnikar et al.) Examiner: M. Lubet
Serial No. 08/617,874)
) Group Art Unit: 1644
Filed: May 21, 1996)
)
For: METHODS AND PRODUCTS)
FOR CONTROLLING IMMUNE) Date: September 27, 1999
RESPONSES IN MAMMALS)

DECLARATION OF ANTHONY M. JEVNIKAR

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Anthony M. Jevnikar, do hereby declare and say as follows:

1. I am presently the Director of the Department of Transplantation Nephrology, London Health Sciences Centre, in London, Ontario, Canada. I am also Associate Professor in the Faculty of Medicine, Department of Microbiology and Immunology, in the University of Western Ontario. A copy of my curriculum vitae is attached as Exhibit 1 to this Declaration.

2. I am a co-inventor named in the above-identified application and have read and understood the Office Action mailed on April 10, 1998 and the Advisory Action mailed on December 11, 1998.

3. The Examiner has rejected claims 19-21, 28, 31-36, 42 and 53-55 as unpatentable over U.S.P. 5,484,719 (Lam et al.), in view of U.S.P. 5,475,086 (Tobin et al.). The Examiner has also rejected claims 1, 5-10, 19-21, 28, 31-36, 41-42 and

53-55 as unpatentable over U.S.P. 5,643,868 (Weiner et al.) and Zhang et al. (P.N.A.S., 88, 10253-10256) in view of U.S.P. 5,484,719 (Lam et al.) and U.S.P. 5,475,086 (Tobin et al.).

4. Lam et al. discloses the production of oral vaccines by expressing antigens characteristic of pathogenic organisms such as hepatitis B virus in plants. Oral administration of such transgenic plants gave stimulation of an immune reaction against the expressed antigen in the subject receiving the vaccine. Stimulation of an immune response is the opposite of inducing immune tolerance, as achieved by the subject invention.

5. It was known prior to the priority date of the present application, September 21, 1993, that plant proteins were glycosylated differently from mammalian proteins. If plant-expressed foreign proteins were also glycosylated differently from normal, or were otherwise slightly altered, this might potentially increase their antigenicity. Such a change would be beneficial if one's aim, as in Lam et al., was to stimulate an immune reaction. It was not known at that date, and it was not predictable, whether the glycosylation pattern of mammalian antigenic proteins would be affected by expression in a plant system or whether an antigen so produced would be effective to induce oral tolerance in a mammal.

6. I have reviewed U.S.P. 5,475,086 (Tobin et al.) and its teachings, at columns 8 to 10, regarding therapy of autoimmune diabetes. In column 8, lines 31 to 55 and column 9, lines 1 to 25, the authors describe the identification of a polypeptide region common to the amino acid sequences of human GAD_{65}, human GAD_{67} and the P2-C protein of coxsackie virus. They postulate that "molecular mimicry" plays a role in the development of autoimmune diabetes, in that after infection with coxsackie virus and activation of T cells to recognise this common polypeptide region, the activated T cells then give rise to an immune response mounted against GAD proteins in the subject's β cells, thus destroying the β cells and causing

diabetes. They suggest that treatment with a polypeptide having the sequence of this common region will block this undesired immune response and prevent disease development (column 9, lines 26 to 46).

This teaching does not suggest that oral administration of plant-expressed, transgenic GAD protein, or even of GAD protein, could prevent the development of autoimmune diabetes.

7. An alternative therapy is proposed at column 9, line 47 to column 10, line 22. The authors suggest stimulation of T-suppressor cells "to restore self-recognition" and ameliorate the disease, by administering a bi-specific antibody, specific both for an epitope of the autoimmune antigen and for an epitope present on the $CD8^+$ receptor.

Again, this teaching in no way suggests that the oral administration of a plant material containing plant-expressed GAD will induce oral immune tolerance, which is not dependent on $CD8^+$ T cells, nor on the ability to cross-link GAD peptides, within or without MHC proteins which might contain them, to T cell receptors. The production of antibodies to GAD is not required for the induction of oral immune tolerance by feeding plant material containing GAD.

8. Finally, it is suggested that polypeptide analogs can be designed "which will compete for recognition of self-antigens at the level of antigen presentation", but "will not activate disease-causing T-helper cells" (column 9, lines 60 to 67). I interpret this to be a suggestion that one can devise analogs of the common polypeptide sequence described by these authors, as discussed in paragraph 9 above, by modification of the amino acid sequences shown in Table 1, as further discussed through to column 11, line 19.

9. None of the therapeutic approaches proposed by Tobin et al., as discussed in paragraphs 6 to 8 above, would, if followed, have led to any appreciation that autoimmune diabetes could be prevented in a susceptible subject by oral

administration of plant material containing plant-expressed GAD protein, as described in the present application. I consider that Tobin et al. teaches away from the present invention, in that the therapeutic approaches suggested would not, in my opinion, lead to oral immune tolerance to an autoantigen such as GAD.

10. I do not believe that the teachings of Lam et al. regarding stimulation of a subject's immune response by administration of plant-produced antigens can be combined with the teachings of Tobin et al., as discussed above, to arrive at the invention described in the present invention.

11. Weiner et al. and Zhang et al. described feeding a native mammalian autoantigen, insulin, to mammals susceptible to autoimmune diabetes to produce oral tolerance to the autoantigen and suppression of the development of the disease.

12. Prior to the work of the present inventors, no one had suggested suppressing or reducing the immune response of a mammal to a mammalian transplantation antigen or autoantigen by feeding to the mammal a plant material obtained from a transgenic plant expressing the relevant transplantation antigen or autoantigen. As noted above, in paragraph 5, it was not known prior to the priority date of this application, and it was not predictable, whether a mammalian antigenic protein would be produced with complete fidelity in a plant-expression system, for example with respect to glycosylation patterns, or whether an antigen so produced would be effective to induce oral tolerance in a mammal. There is nothing in the teachings of Weiner et al. or Zhang et al. to give any guidance in this regard.

13. The present inventors have found that plant material obtained from a transgenic plant expressing a mammalian autoantigen can be used to produce oral immune tolerance, as described in the application and further described in Nature

Medicine (1997), v. 3, p. 739, a copy of which was filed with Applicant's response dated January 5, 1998.

14. Furthermore, the inventors have found unexpectedly that plant material containing plant-expressed mouse GAD protein stimulated a greater proliferative response of GAD-primed T cells than highly purified recombinant mouse GAD expressed in E.coli. This study is described in greater detail in Exhibit 2 attached. These findings indicate that plant material containing plant-expressed transgenic GAD gave enhanced T cell activation, which is a pre-requisite step in the induction of immune tolerance, including oral tolerance.

15. Such enhancement may be related to altered glycosylation of the antigen when expressed in plants; it has been shown that the glycosylation of an allergen influence the affinity of antibodies against that allergen (Batanero et al., (1994), Molecular Immunology, 31, 31). Similarly, the processing, transport and binding of antigen fragments to MHC molecules on antigen-presenting cells, which plays a central role in oral tolerance, will be affected by the glycosylation pattern of the antigen.

16. Additionally, oral administration of plant material obtained from a transgenic plant expressing a mammalian transplantation antigen or autoantigen results in administration not only of the recombinant antigen but also of additional plant components which may assist in the induction of oral immune tolerance. For example, plant lectins are bound to nucleated cells of the gut and lectins are known to arrive intact in the small intestine, where there are lymphoid cells involved in the induction or oral tolerance.

Lectins bound to gut cells can be endocytosed by the gut epithelium, where they can have a direct effect on the epithelial cells and, by acting as growth factors, can affect the immune response (Pusztai, (1993), Eur. J. Clin. Nutrition, 47, 691). As one example, Vehmeyer et al.,(1998), Eur. J. Haematol., 60, 16, has recently

shown that the plant lectin VAA-1 binds to haematopoietic cells, including CD34+ progenitor cells which are represented in the gut, and has a co-stimulatory effect on proliferation of such cells in humans. Many other lectins are known to exist in plant cells.

As noted above, T cell activation is required in order to produce oral tolerance.

17. The teachings of Lam et al. and Tobin et al. cannot, in my opinion, be combined to arrive at the present invention, as discussed in paragraph 10 above; the teachings of Weiner et al. and Zhang et al. regarding use of a native mammalian autoantigen do not supplement the teachings of Lam and Tobin so as to arrive at an appreciation that plant material containing plant-expresssed mammalian antigens could be administered orally to produce immune tolerance.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardise the validity of the application or any patent issued thereon.

A. M. Jevnikar
Anthony M. Jevnikar

Sept 29/99
Date